

In response to Applicants' previous arguments that the apparatus components required are commonly used in chemiluminescence devices and the previously submitted drawings from technical manuals of a representative chemiluminometer, autosampler, and HPLC pump, the Examiner argues that there is still inadequate evidence to establish that the claimed components are commonly used in chemiluminescence devices and are well known in the art. The Examiner takes the position that the devices of the drawings merely show outside housings and provide no description of the devices, and that it is unclear when these technical manuals were published and available to the public. The Examiner suggests that articles and/or patents that are readily available to the public and published prior to the filing date of the present application should be supplied which describe components and functioning of the chemiluminescence devices required by the claims.

Applicants respectfully traverse this rejection and the arguments in support thereof for the reasons set forth previously on the record, which Applicants rely upon in full, and for the additional reasons which follow, and respectfully request reconsideration and withdrawal of the rejection.

As previously explained on the record, the presently claimed invention is directed to a novel and quantitative chemiluminescence system using a flow cell packed with immobilized horseradish peroxidase (HRP) for determining the concentration of hydrogen peroxide in body fluid. The presently claimed device contains two mobile phase passages, two chromatography pumps, an autosampler, a joined flow passage, and a chemiluminometer having a flow cell in which a HRP immobilized stationary phase is packed.

In the presently claimed device, an autosampler collects a sample of body fluid, which is then pumped by a first chromatography pump into a first mobile phase passage or tube. A second chromatography pump is used to inject a solution of imidazoles and an alkaline buffer into a second mobile phase passage or tube. These two tubes are then joined together into a single tube, designated as a flow passage, which transports the body fluid and the imidazoles and alkaline buffer solution into the flow cell of a chemiluminometer. In the flow cell, which is packed with a HRP immobilized stationary phase, the body fluid, the imidazoles, and the alkaline buffer are mixed together and react with the immobilized HRP.

In a preferred embodiment, the reaction mechanism of the chemiluminescence, which occurs in the flow cell of the chemiluminometer, first involves the immobilized HRP, the hydrogen peroxide, and dissolved oxygen, which, in the presence of the alkaline buffer, oxidizes the imidazole to imidazole hydroperoxide. The imidazole hydroperoxide is then further oxidized to imidazole dioxetane, which emits light while decaying. The intensity of the light emitted by this reaction is measured by a photomultiplier of the chemiluminescence detecting system.

As will be appreciated by one skilled in the art, the presently claimed invention is essentially directed to the use of liquid chromatography to detect a compound, hydrogen peroxide, based on the chemiluminescence reaction which takes place in the flow cell. The novel component of the presently claimed device is a flow cell in which a HRP immobilized stationary phase is packed, thereby utilizing HRP as the light emitter.

As shown in Fig. 1 and described at page 3, lines 24-27 of the application, the presently claimed invention uses two high performance liquid chromatography pumps 11, 21, an autosampler 12, and a chemiluminometer 31 with a flow cell 32. Attached herewith as Appendix 1 is a photograph of a representative experimental set-up showing all of the devices as they might be connected for use. Applicants are using such a set-up to determine hydrogen peroxide as described in the present application. One skilled in the art would clearly be able to replicate such a set-up and method based on the teachings of the application.

The claimed components are all commonly used in chemiluminescence devices and preferred components are described at page 5, lines 16-23 of the specification. Enclosed herewith as Appendix 2 are technical specification sheets for a representative chemiluminometer (825-CL), autosampler (AS-950), and HPLC pump (PU-980), manufactured by JASCO Corporation. These specification sheets describe the functions and attributes of these instruments. Applicants acknowledge that these documents have no publication date. However, these specific instruments are no longer being manufactured by JASCO Corporation. Applicants note that technical specification sheets for currently manufactured analogous instruments are readily available to the public from the JASCO Corporation website and, when the devices described in the application were being manufactured, their manuals were similarly readily available. Applicants have attempted several times to obtain complete (and dated) technical

manuals for the 825-CL, AS-950, and PU-980 instruments. However, several representatives from the JASCO Corporation have evidently been unable to provide Applicants with such manuals. Further, it is noted that the presently claimed invention is not limited to the specific devices described in the application. Rather, it would also be within the scope of the invention to utilize any known or to be developed chromatography pump, chemiluminometer, autosampler, etc.

As further evidence that the representative devices (825-CL, AS-950, and PU-980) were available at the time the present application was filed (August 27, 2003), Applicants enclose herewith as Appendix 3 several journal articles and patents, published or issued prior to August 27, 2003, which discuss the use of one or more of these instruments. For example, U.S. Patent No. 5,814,742 of Vissers (September 29, 1998) describes in col. 4, line 17 the use of a PU-980 HPLC pump, and U.S. Patent No. 6,262,311 of Maassen (July 17, 2001) teaches obtaining HPLC measurements using a system including a PU-980 pump and an AS-950 automatic sampler. A literature article by Wadano et al. (*Agric. Biol. Chem.*, **55** (5), 1217-1223 (1991)) describes at pages 1218-1219 a chemiluminescence detector system and the use of a JASCO 825-CL detector. Detsi et al. (*J. Chem. Soc., Perkin Trans. 1*; 2443-2449 (1998)) describes at page 2446, second column, the use of an HPLC system containing a PU-980 pump and an AS-950 autosampler. Finally, Tsunoda et al. (*Analyst*; **126**, 637-640 (2001)) describe an HPLC chemiluminescence reaction detector system including a PU-980 HPLC pump and an 825-CL chemiluminescence detector (page 638, first column). Accordingly, it is apparent that such devices were commonly used in the art at the time the present application was filed. These citations describing various chemiluminescence and spectroscopic systems clearly demonstrate, as requested by the Examiner, that the components of the claimed device were indeed commonly used in chemiluminescence devices and were well known in the art at the time of the invention.

Finally, enclosed herewith as Appendix 4 is a literature article by Nakane (*Journal of Histochemistry and Cytochemistry*; **22**(12); 1084-1091 (1974)) which is referred to in paragraph [0026] of the present application publication. This article, published prior to the filing of the present application, describes the immobilization of horseradish peroxidase into the stationary phase. Accordingly, it can be clearly seen that this claimed element was well known in the art at the time of the invention.

In conclusion, as noted above, the presently claimed device is not limited to the exemplified instruments or devices. Rather, since all of the aforementioned components are commonly used devices in an apparatus for chromatographic and chemiluminescence analysis, their structure, operation, and functionality are commonly known to one skilled in the art and, as such, the apparatus structure of the presently claimed device is readily understandable by one skilled in the art. Accordingly, reconsideration and withdrawal of the § 112, first paragraph rejection are respectfully requested.

Based on the preceding remarks and Appendices submitted herewith, it is respectfully submitted that the pending claims are in full compliance with § 112 and in condition for allowance. A Notice of Allowance is respectfully requested.

Respectfully submitted,

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Encl: Appendix 1: Photograph of exemplary experimental setup

Appendix 2: Technical Specifications of JASCO Chemiluminometer, Autosampler, and HPLC Pump

Appendix 3: U.S. Patent No. 5,814,742 of Vissers; U.S. Patent No. 6,262,311 of Maassen; Wadano, et al. (*Agric. Biol. Chem.*, **55** (5), 1217-1223 (1991); Detsi et al. (*J. Chem. Soc., Perkin Trans. 1*; 2443-2449 (1998); Tsunoda et al. (*Analyst*, **126**, 637-640 (2001).

Appendix 4: Nakane (*Journal of Histochemistry and Cytochemistry*; **22**(12); 1084-1091 (1974))